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The authors wish to dedicate this paper to the memory of Professor Irena Mochnacka (1905 -1979), the Editor-in-Chief of Acta Biochimica Polonica

Nonidentity of subunits of human kidney arginase A1 and human liver arginase A5

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The main forms of arginase A1 from human kidney and A5 from human liver were purified to homogeneity. Molecular weight of both forms of enzyme approximates 120000. In the presence of EDTA these arginases dissociate into single type distinct subunits. M_r of both kinds of subunits is 30000. Similarly as native arginase forms, they differ in electric charge and display complete immunological incompatibility.

The oligomeric structure of mammalian liver arginase is well documented. Hirsch-Kolb & Greenberg [1] were the first who reported that a homogeneous preparation of the main form of rat liver arginase dissociates in the presence of 8 M urea into single type of subunits M_r 30000. The subunit structure has been also demonstrated in human [2], rat [3], rabbit [4], dog, ox, pig and mouse [5] liver arginases.

Almost all of the mammalian liver arginases are considered to be of a tetramer [1] or trimer [5, 6] structure, built of M_r 30000 - 45000 subunits.

Whereas the heterogeneity of liver arginase is now generally accepted, the subunit structure of arginases from other organs has not been studied. There are only a few reports stating that antisera against human and rat liver arginase precipitate liver arginases from different mammalian species but not the arginases from the kidney [5, 7]. The immunological difference between mammalian arginases from liver and kidney is usually postulated on the basis of lack of cross-reaction of the kidney arginase with the serum against arginase A5. Moreover, so far it has not been taken into account that two forms of the enzyme occur both in liver and kidney [8, 9].

The serum against kidney A1 arginase obtained at our laboratory [8] contributed to the possibility of differentiation between the multiple forms of arginase from various mammalian tissues. In an earlier report from our laboratory the presence of five arginase forms in rat tissues has been reported [8]. All these five forms have an identical M_r of 120000, and upon treatment with EDTA they dissociate into subunits of M_r 30000. In the presence of Mn^{2+} ions the subunits reassociate to the native forms of the enzyme of M_r 120000. The main liver arginase form A5, and the main kidney arginase form A1 are characterized by entirely different charge, as they are anodic and cathodic proteins, respectively. These two forms show complete immunological incompatibility in the presence of two kinds of sera: anti-A5 and anti-A1, whereas arginases A2, A3 and A4 display partial immunological similarity both with respect to each other and to arginases A1 and A5 [8].

The use of the anti-A1 serum in addition to the commonly applied anti-A5 serum, allowed to

distinguish more precisely the five arginase forms from rat [8] and human [9] tissues. Only the application of the two antisera together allowed to demonstrate the complete immunological incompatibility between the native forms of A1 and A5. Since they both have an oligomeric structure, and each of them is built of distinct single-type subunits, it could be assumed that these subunits may also display complete immunological incompatibility.

In the present work we report on some features of the subunits of arginase A1 from kidney, and we compare them with those of the subunits of arginase A5 from human liver.

MATERIALS AND METHODS

Reagents. Reagents were purchased as follows: L-arginine (Calbiochem, Los Angeles, Cal. U.S.A.), Sephadex G-150, Dextran 2000, DEAE-Sephacel (Pharmacia, Uppsala, Sweden), CM-cellulose CM-2 and DEAE-cellulose DE-11 (Whatman Biochemicals Maidstone, Kent, England), Freund's incomplete and complete adjuvant (Difco Laboratories, Detroit, Michigan, U.S.A.). Bovine albumin, chicken ovalbumin, bovine γ-globulin and horse myoglobulin (Sigma Chemicals Co., St. Louis, Mo, U.S.A.). All other chemicals were of the purest available grades from standard commercial sources.

Materials. Human liver and human kidney were taken within 20 - 30 h after death of persons 25 - 45 years old, killed in traffic accidents, with no pathological changes observed on autopsy.

Arginase assay. Arginase activity was measured by determining the increase in the amount of the reaction product, ornithine [10]. One unit (U) of enzymic activity was defined as 1 μmole of the product formed per 1 min at 37°C.

Protein determination. Protein was assayed according to Lowry *et al.* [11] or spectrophotometrically with crystalline bovine serum albumin as a standard [12].

Molecular weight determination. The M_r of human erythrocyte arginase was determined by Sephadex G-150 chromatography [13]. The column (2 × 42 cm) was equilibrated with 100 mM KCl in 50 mM Tris/HCl buffer, pH 7.5. Fractions of 2 ml were checked for enzymic activity and protein content. Horse myoglobulin M_r 17000, ovalbumin M_r 46000, bovine serum albumin M_r 69000 and bovine serum γ-globulin M_r 150000 were used as standards.

Purification of antigens. Arginase A1 from human kidney and arginase A5 from human liver were purified according to the procedure described by Skrzypek-Osiecka *et al.* [14] and by Ber and Muszyńska [15], respectively. Pure arginase A1 (sp. act. 1000 μmol × min^{-1} per mg protein) and pure arginase A5 from human liver (sp. act. 2500 μmol × min^{-1} per mg protein) was raised in female guinea pigs.

Preparation of antiserum, double immunodiffusion and immunoelectrophoresis were carried out as described previously [8].

Preparation of subunits of arginase A1 from kidney and subunits of arginase A5 from liver. Pure human arginase A1 and pure human arginase A5, 2 mg and 4 mg of protein, respectively, were dialyzed against 50 mM Tris/HCl buffer, pH 7.5 and incubated with 30 mM EDTA at 37°C for 30 min. Molecular weight was determined by filtration on Sephadex G-100 column equilibrated with the solution containing 10 mM EDTA and 100 mM Tris/HCl buffer, pH 7.5. Peptides of M_r 30000 obtained from arginase A1 and arginase A5 are further referred to as subunit K (kidney) and subunit L (liver), respectively.

RESULTS

Molecular weight

M_r of the native pure arginase A1 as well as that of the native pure arginase A5 is 120000 (for both forms of enzyme) as estimated by gel filtration on Sephadex G-150. M_r of subunits K of arginase A1 and of subunits L of arginase A5, obtained after EDTA treatment, was found to be 30000.

Electrophoretic properties

On polyacrylamide gel electrophoresis the native form of arginase A1 migrates as a single protein band towards the anode (Fig. 1 c). A5 exhibits low electrophoretic mobility remaining near the cathode (Fig. 1 a), under the conditions used. The subunits K and L (M_r 30000) migrate also as distinct protein bands towards the anode and cathode, respectively, like their parental native forms (Fig. 1 d, b).

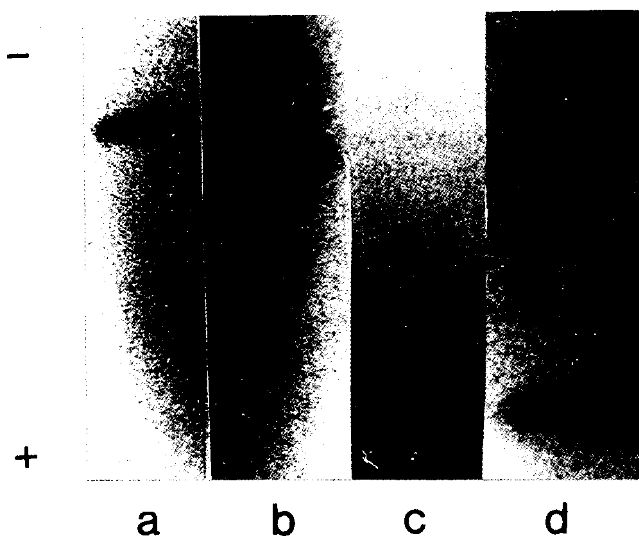

Fig. 1. Polyacrylamide slab gel electrophoresis.
Electrophoresis was carried out in 7% w/v polyacrylamide, using Tris/glycine buffer, pH 8.9. Protein was stained with Coomassie brilliant blue R 250. Native arginase A5 (a) and native arginase A1 (c), as well as, subunits L (b) and subunits K (d) (5 µg and 7 µg of protein, respectively) were applied.

Immunological properties

In the double immunodiffusion test arginase A1 from human kidney reacts with anti-A1 serum (Fig. 2 Ia), but shows no cross-reaction with the serum against arginase A5 from human liver (Fig. 2 Ib). Similarly, arginase A5 from human liver forms a precipitation line only with the anti-A5 serum (Fig. 2 IIa). No cross-reaction is found with the serum against arginase A1 (Fig. 2 IIb).

The subunits of either arginase form retain the ability to react with antibodies, similarly as the native forms A1 and A5 and react only with the antisera prepared against their respective parental arginase forms (Fig. 3 I, II).

When both types of subunits K and L (Fig. 4 I) and their respective native enzyme forms A1 and A5 (Fig. 4 II), are tested in the presence of both types of antiserum simultaneously, they produce precipitation arcs crossing each other. This pattern shows that arginase A1 from the kidney and arginase A5 from the liver, as well as their respective subunits, are completely in-

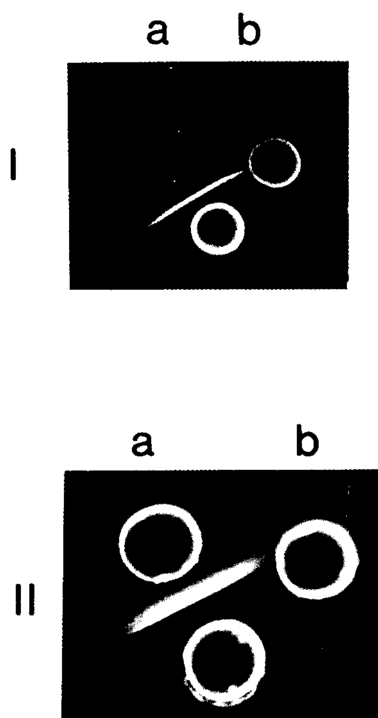

Fig. 2. Double immunodiffusion of native arginase A1 and arginase A5.
Centre well: I, serum against arginase A1; II, serum against arginase A5; Ia, IIb, arginase A1; Ib, IIa, arginase A5.

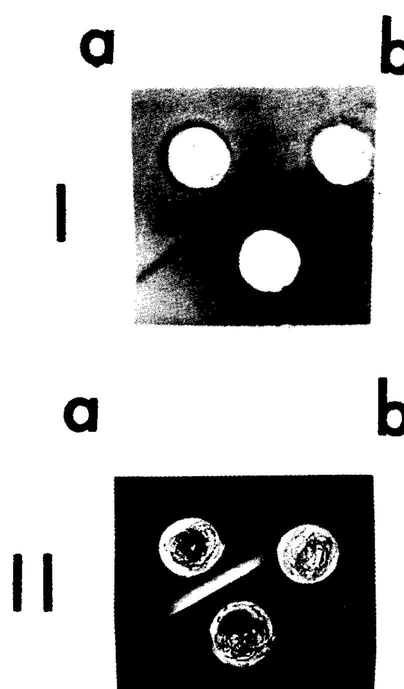

Fig. 3. Double immunodiffusion of separated subunits K and subunits L.
Centre well: I, serum against arginase A1; II, serum against arginase A5; Ia, IIb, subunits K; Ib, IIa, subunits L.

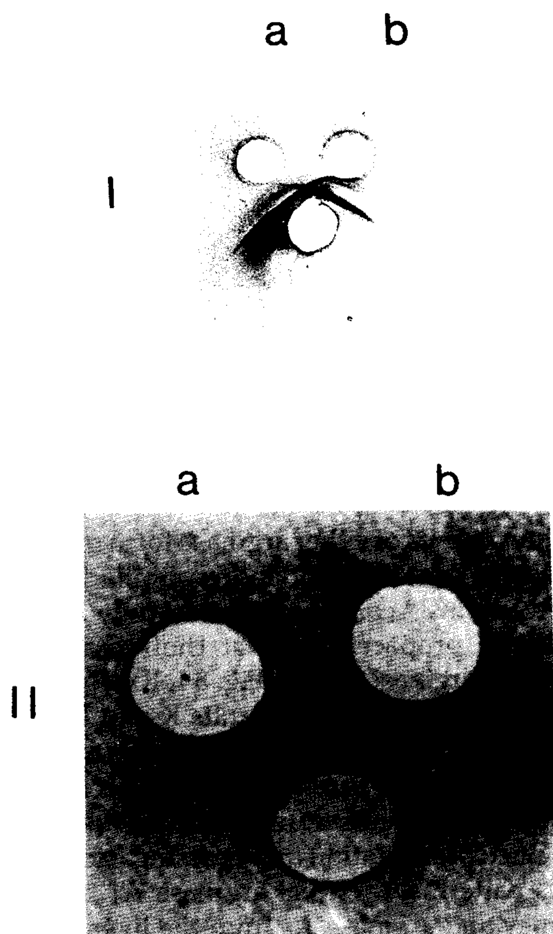

Fig. 4. Immunological relationships between the separated subunits K and L, and native arginases A_1 and A_5.
Centre well: I, subunits L and subunits K; II, native arginase A_1 and A_5; Ia, IIb, serum against arginase A_1; Ib, IIa, serum against arginase A_5.

compatible immunologically and have different antigenic determinants.

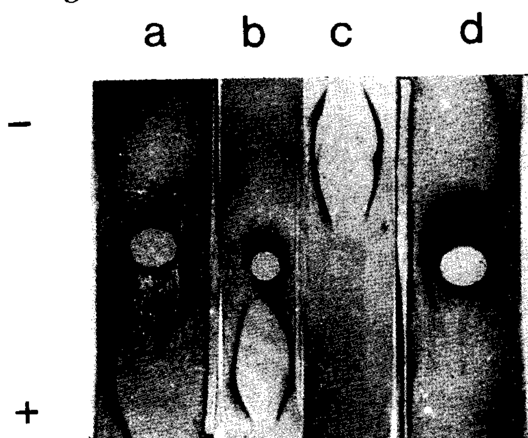

Fig. 5. Immunoelectrophoresis of separated subunits K and L and of native arginase A_1 and A_5.
a, Subunits K; b, arginase A_1; c, arginase A_5; d, subunits L. Cross-reaction developed in the presence of a mixture of sera against arginase A_1 and against arginase A_5.

Figure 5 illustrates the immunoelectrophoretic properties of arginase A_1 and arginase A_5, as well as those of the subunits of both forms. The kidney arginase A_1 composed of anionic subunits and the liver arginase A_5 made up of cationic subunits exhibit cross-reaction arcs near to anode and cathode, respectively (Fig. 5 b, c). The K and L subunits of M_r 30000 display the same direction of migration as their native forms (Fig. 5 a, d).

DISCUSSION

Analysis of the subunit structure of arginase is of great interest. It is a prerequisite for better insight into the biosynthesis, distribution, regulation and physiological function of different forms of arginase in distinct mammalian tissues. Knowledge of molecular structure of arginase may facilitate further studies on its hormonal and age-related regulation, as well as can elucidate the molecular basis of hiperargininemia caused by a deficiency of liver arginase.

In the present studies on a pure arginase preparation we established that each of two investigated arginases, A_1 from human kidney and A_5 from human liver, is composed of a single type subunits, K and L, respectively. Both arginases are composed of the same molecular weight subunits (M_r 30000) but similarly as their native forms, they differ greatly in electric charge and show complete immunological incompatibility. These results indicate that in human tissues, kidney arginase A_1 and liver arginase A_5, are parental forms, each built of single-type subunits, K and L, respectively. Recombination of these subunits in various proportions produces the remaining three forms of arginase A_2, A_3 and A_4 found in other tissues. The quantitative relation of the subunits K and L in these three forms has not so far been determined. However there is evidence that each of them is a hybrid of these subunits. Namely, Porembska *et al.* [8, 9] have shown that, in contrast to forms A_1 and A_5 which give cross-reactions only with the respective antibodies, the other three forms: A_2, A_3, A_4, precipitate both with antibodies against kidney arginase A_1 and liver arginase A_5. The hybrid nature of the forms A_2, A_3 and A_4 has been confirmed by immunoelectro-

phoresis of an EDTA-treated preparation of arginase A_3 from rat submaxillary gland [8].

It is noteworthy that only in the rat, cat, dog and humans the form A_5, built exclusively of subunits L, is the main liver arginase [16]. In contrast, in the ox, horse, calf and pig the main liver arginase consist of form A_2, being a hybrid of the subunits K and L. These results explain the report of Brusdeilins *et al.* [5] that bovine and human liver arginases are not identical. According to our results, this is due to the difference between the bovine and human liver arginase in the subunit structure; it is also possible that there are some species-dependent differences between ox and humans in the subunit gene.

In our earlier studies [16] we have found that the pattern of distribution of the arginase forms in the liver and kidney of mammals is rather tissue-dependent than species-dependent. The presence of arginases A_5 and A_2 is characteristic of the liver, and that of the forms A_1 and A_4 – of the kidney. It is of interest that in all of the investigated mammals the arginase A_1 is always the main form in the kidney, whereas the hybrid A_4 occurs in a very small amount. These relation are not so regular in liver arginase as the amounts of form A_5 and hybrid form A_2 vary from species to species.

The complete immunological incompatibility between the subunits K and L may testify to their dissimilar first-order structure, and perhaps also to their being coded by different genes. Upon analysis of arginase distribution in patients with argininemia caused by arginase deficiency, Spector *et al.* [7] have concluded that there are two gene loci coding for the distinct human liver and kidney enzymes.

This conclusion is also supported by Dizikes *et al.* [19, 20] who have shown that cDNA for liver arginase does not hybridize with the genetic material of kidney arginase. A group of Japanese investigators [19, 20] demonstrated the occurrence of a distinct arginase mRNA species of about 1600 nucleotides in rat liver, but not in rat kidney, spleen, heart or small intestine.

Whereas the studies on the identification of gene of rat and human liver arginase are well advanced, no adequately documented evidence for the existence of a distinct gene for kidney arginase has so far been obtained.

To answer the question whether subunits L and K are the products of two genes further investigations are needed.

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